CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20828

CHEMISTRY REVIEW(S)

DRAFT

DIVISION OF ANTIVIRAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-828

CHEMISTRY REVIEW #: 1 **DATE REVIEWED**: 31-Oct-97

SUBMISSION	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	09-May-97	12-May-97	14-May-97
Amendment	01-Jul-97	03-Jul-97	22-Jul-97
Amendment	09-Jul-97	10-Jul-97	22-Jul-97
Amendment	21-Jul-97	22-Jul-97	25-Jul-97
Amendment	30-Jul-97	01-Aug-97	04-Aug-97
Amendment	28-Aug-97	29-Aug-97	04-Sep-97
Amendment	15-Oct-97	17-Oct-97	24-Oct-97
Amendment	22-Oct-97	23-Oct-97	27-Oct-97

NAME & ADDRESS OF SPONSOR: Hoffmann-La Roche Pharmaceuticals Inc.
340 Kingsland Street
Nutley, N.J. 07110

DRUG PRODUCT NAME

Proprietary: FORTOVASE®
Nonproprietary: Saquinavir
Code Name/#: Ro 31-8959; CAS # 127779-20-8
Chem. Type/Ther. Class: 2 P

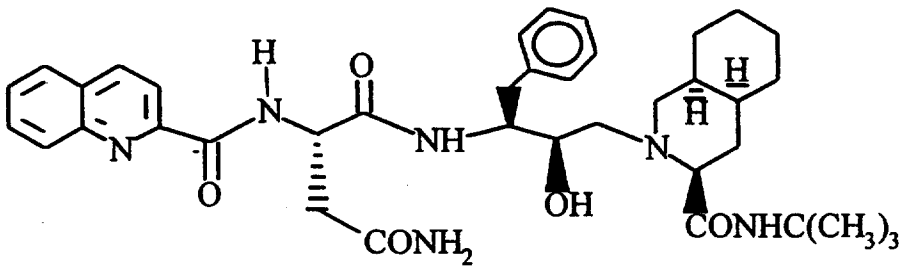
PHARMACOLOGICAL CATEGORY: Antiviral: Anti-HIV

INDICATION: Treatment of HIV infection.

DOSAGE FORM/STRENGTH: Capsules Soft Gelatin, 200 mg

ROUTE OF ADMINISTRATION: Oral

CHEMICAL NAME/STRUCTURAL FORMULA:
N-*tert*-Butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aS, 8aS)-isoquinoline-3(S)-carboxamide
($C_{38}H_{50}N_6O_5$; Mol. Wt. 670.)



Saquinavir

SUPPORTING DOCUMENTS:

RELATED DOCUMENTS:

Chemistry Reviews of IND
Chemistry Review of NDA 20-628.
Record of facsimile of Sep-29-97 (CMC comments and requests regarding drug substance & drug product).

CONSULT REVIEWS:

Review of Tradenames (CDER Labeling and Nomenclature Committee, Consult # 837).
Environmental Assessment (N. Sager, HFD-357).

CONCLUSIONS & RECOMMENDATIONS:

The NDA submission and accompanying amendments provided adequate information on the chemistry, manufacturing and controls for FORTOVASE. The related evaluation of the manufacturing facilities has been completed and is satisfactory. The Environmental Impact Analysis is also acceptable. The NDA, as amended, is therefore recommended for **approval** from a chemistry standpoint.

Paul S. Liu, Review Chemist

Concurrence:
HFD-530/SMiller

cc:
Orig. NDA 20-828
HFD-530/Div. File
HFD-530/Dep. Div. Director
HFD-530/SMiller
HFD-830/Div. Director
HFD-530/PLiu
HFD-530/TNguyen
HFD-530/KStruble
HFD-530/NBattula
HFD-530/KWu
HFD-530/CKelly
File: N-20828.000

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20828

ENVIRONMENTAL ASSESSMENT AND/OR FONSI

ENVIRONMENTAL ASSESSMENT

AND

FINDING OF NO SIGNIFICANT IMPACT

FOR

SAQUINAVIR

SOFT GELATIN CAPSULES

NDA 20-828

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

DIVISION OF ANTIVIRAL DRUG PRODUCTS
(HFD-530)

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-828

Saquinavir

Soft Gelatin Capsules

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process. FDA's review focuses on the relevant environmental issues relating to use and disposal from use of FDA-regulated articles.

The Food and Drug Administration, Center for Drug Evaluation and Research has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for **Saquinavir Soft Gelatin Capsules, Hoffmann-La Roche Inc.** has conducted a number of environmental studies and prepared an environmental assessment (attached) in accordance with 21 CFR § 25 which evaluates the potential environmental impacts of the proposed action.

Saquinavir is a synthetic drug which is administered as an oral capsule in the treatment of Acquired Immunodeficiency Syndrome (AIDS) and AIDS-Related Complex (ARC). The finished drug product will be used in hospitals, clinics and by patients in their homes. Because of the quantity of drug expected to be used in the United States, the relevant environmental issue relating to this action is whether saquinavir entering the environment from consumer use and disposal will adversely affect environmental organisms.

Chemical and physical test results indicate that the drug entering the environment may exist in the aquatic or terrestrial environments. No rapid environmental depletion mechanism has been identified, although the compound is expected to bind tightly to soils and sediments thus limiting its bioavailability to environmental organisms. As saquinavir is expected to persist in the environment for some time, the toxicity of the material to organisms was characterized. Studies were conducted to assess the acute toxicity to water fleas *(Daphnia magna)*, rainbow trout *(Oncorhynchus Mykiss)*, green algae *(S. capricornutum)*, the

subacute toxicity to earthworms (*Lumbricus terrestris*) and the inhibitory effect on microbial growth and activated sludge respiration. These studies indicate that there are no expected adverse environmental effects at the expected environmental concentrations. The estimated concentration of the active moiety, saquinavir, entering the terrestrial or aquatic environment is more than 3 orders of magnitude lower than the toxicity test results.

At U.S. hospitals and clinics, empty or partially empty packages will be disposed according to hospital/clinic procedures. From home use, empty or partially empty containers will typically be disposed of by a community's solid waste management system which may include landfills, incineration and recycling, while minimal quantities of unused drug may be disposed of in the sewer system.

The Center for Drug Evaluation and Research has concluded that the product can be used and disposed of without any expected adverse environmental effects.

9/8/97
DATE

PREPARED BY
Nancy B. Sager
Environmental Scientist
Center for Drug Evaluation and Research

9-8-97
DATE

CONCURRED
Eric B. Sheinin, Ph.D.
Director, Office of New Drug Chemistry
Center for Drug Evaluation and Research

Attachment: Environmental Assessment

1. **DATE**

April 29, 1997

2. **NAME OF APPLICANT**

Hoffmann-La Roche Incorporated

3. **ADDRESS**

340 Kingsland Street

Nutley, New Jersey 07110

4. **DESCRIPTION OF THE PROPOSED ACTION**

a. Requested Approval

Approval is being sought to market a new dosage form of saquinavir for the treatment of HIV disease in the United States. The new drug product, TRADE NAME™ (saquinavir) Soft Gelatin Capsules (200 mg) (NDA No. 20-828), will be packaged in high density polyethylene bottles (425 cc), fitted with plastic safety caps (45 mm) over metal screw caps with pulp/polyethylene liners and glassine tacseals. The capsule count per bottle is 180.

The information provided in this Environmental Assessment (EA) updates and augments information given in the EA for NDA No. 20-~~682~~ 628 for Invirase®(saquinavir) Capsules (200 mg), which was approved December 6, 1995. Information from this previous EA is reproduced in appendices of this document wherever applicable, and new information is provided as appropriate, relevant to the use of the base instead of the mesylate salt as the drug substance, new manufacturing sites, and the formulation of the new drug product. The production forecast included in the original EA has been updated to reflect the greater than anticipated demand for the drug product and the increased use of the saquinavir active moiety resulting from consumption of both dosage forms through the anticipated fifth year (2001) following approval of NDA No. 20-828. However, use of the new drug product is expected to replace consumption of the original formulation. The new forecast has been used to

revise the estimated worst-case concentration of saquinavir base that could occur in the aqueous compartment of the environment from use.

The Center for Drug Evaluation and Research (CDER) (November, 1995) has significantly revised the guidance for the preparation of EAs since the submission of NDA No. 20-682. Information not included in the original EA but explicitly required under the new guidance has been added to this document.

The proposed action includes synthesis of the drug substance, formulation of the drug product, preparation and packaging of the capsules, and use and disposition of the product designated in this EA as TRADE NAME™ (saquinavir) Soft Gelatin Capsules (200 mg). The drug substance in this product is saquinavir base (Fig. 4-1).

This EA is part of New Drug Application No. 20-828 for TRADE NAME™ (saquinavir) Soft Gelatin Capsules (200 mg). Its format is arranged as required by 21 CFR §25.31a(a). Supporting documents for the items discussed in this EA have been organized as appendices in Section 15. All confidential material is provided in Appendix H. Cited references are included in Appendix G.

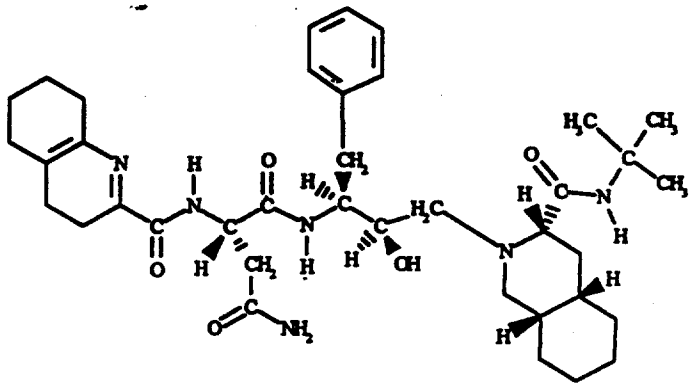
b. Need for Action

NDA No. 20-682 (28) was approved December 6, 1995 for the use of Invirase®(saquinavir) Capsules (200 mg) in the treatment of HIV disease in the United States. Hoffmann-La Roche Incorporated is now seeking approval to market a soft gelatin capsule containing the drug substance in the form of saquinavir base, rather than the mesylate salt, in the same strength (as base) and for the same indication as that approved for the original formulation.

Saquinavir is a synthetic peptide-like substrate analogue of viral polyprotein precursors that inhibits the activity of HIV protease and prevents the cleavage of viral polyproteins. Despite the proven efficacy of the original

Figure 4-1

Saquinavir



hard gelatin capsule dosage form, the formulation is known to have a low and variable absolute bioavailability. This fact, and the results of a clinical study using higher than registered doses of saquinavir, which showed that greater, more durable effects on HIV-RNA and CD_4 cell count could be achieved with increased drug exposure, indicated the necessity of identifying an improved formulation with increased bioavailability. A number of pilot formulations were studied, leading to the development of a soft gelatin capsule formulation with a saquinavir bioavailability in the region of 300% relative to the registered drug product.

c. Locations of Production

Saquinavir base, the drug substance, is manufactured by Hoffmann-La Roche Ltd., Grenzacherstrasse 124, CH-4070 Basle, Switzerland. The drug product, TRADE NAME™ (saquinavir) Soft Gelatin Capsules (200 mg) will be manufactured by R.P. Scherer GmbH, Gammelsbacher Strasse 2, 69412 Eberbach/Baden, Germany, and shipped in bulk to Hoffmann-La Roche Incorporated, 340 Kingsland Street, Nutley, New Jersey, 07110, where it will be packaged and labeled.

Although Hoffmann-La Roche Ltd. is responsible for the final steps in the synthesis of saquinavir base, the manufacture involves multiple companies, Roche Group sites, and alternate and additional sites throughout Europe and the United States. Synthesis (Confidential Appendices (H-1 and H-2) is divided into three parts: Part A is the synthesis of and Part B is the synthesis of ' In Part C, ' are covalently linked and additional synthesis is carried out to obtain the drug substance. On October 23, 1995, prior to the approval of NDA No. 20-682 but after review of the EA, an amendment was submitted documenting an additional route of synthesis, whereby the proprietary intermediate is substituted for in Part C, synthesis is carried out to obtain the drug substance. Concurrently, an agreement was reached with Dr. Paul Liu, Review Chemist in the Office of New Drug Chemistry, that the intermediates which ordinarily would be considered proprietary intermediates under current Chemical Manufacturing and Controls regulations and guidance, would be treated as starting materials. The Memo of Record documenting this agreement is reproduced in Appendix A. Consistent with this agreement, the two intermediates are treated as starting materials in this EA, and therefore are not discussed to a significant extent. However, all required information relevant to their previous status as proprietary intermediates was included in the EA for NDA No 20-682. For detailed descriptions of the original and additional routes of synthesis, please see the flow charts in Confidential Appendices H-1 and H-2, respectively.

Manufacturers of Proprietary Intermediate and the Drug Substance:

For brief descriptions of the environments at and adjacent to the U.S. facilities, including the manufacture of the proprietary intermediate and the packaging of the drug product, please see Appendix B.

d. Locations of Use and Disposal

As a prescribed medication for the treatment of HIV disease, this drug is ingested and eliminated wherever the patients spend their day. The updated production forecast for the saquinavir active moiety, reflecting the use of both TRADE NAME™ (saquinavir) Soft Gelatin Capsules (200 mg) and Invirase®(saquinavir) Capsules (200 mg) (soft gelatin and hard gelatin capsule dosage forms) during the fifth year following approval (2001) is expressed in terms of kilograms of saquinavir base in Confidential Appendix H-3. The amount that is eliminated (or excreted) will enter municipal treatment systems throughout the United States. At the Hoffmann-La Roche facility in Nutley, process and washdown water are sewered through a pretreatment system to the

Passaic Valley Sewerage Commission (PVSC), a publicly owned treatment works. No aqueous wastes are anticipated to be discharged as a result of the drug product packaging and labeling operations at Nutley. All solid waste, including waste packaging, off-specification and returned expired drug product, drug product used for laboratory testing or cleaned from machinery, fabric dust collectors, and protective clothing worn by operators will be collected for incineration on-site. Please see Appendix C for information pertinent to this operation.

At U.S. hospitals, pharmacies or clinics, empty or partially empty bottles will be disposed of according to hospital, pharmacy or clinic procedures. In the home, empty or partially empty containers will typically be disposed of by a community's solid waste management system which may include landfills, incineration and recycling, although minimal quantities of unused drug may be disposed of in the sewer system.

5. IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

a. Nomenclature

i. United States Adopted Name (USAN)

saquinavir

ii. Brand/Proprietary Name

TRADE NAME™

iii. Chemical Name

cis-N-*tert*-Butyl-decahydro-2[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxyamide

b. CAS Registry Number

127779-20-8

c. Molecular Formula

$C_{38}H_{50}N_6O_5$

d. Molecular Weight

670.855

e. Structural (graphic) Formula

Please see Figure 4-1, page 3.

f. Physical Description

Saquinavir is a white fine powder with possible lumps. Its chemical and physical properties are listed in Table 5-1. A Material Safety Data Sheet (MSDS) for the mesylate salt of drug substance is in Appendix D.

g. Additives and Impurities

The quantitative composition of TRADE NAME™ (saquinavir) Soft Gelatin Capsules (200 mg), with CAS Registry numbers, is provided in Confidential Appendix H-4. Under current specifications, in each acceptable batch of saquinavir base, the sum of all impurities is ≤ 1.0 percent.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

a. Substances Emitted During Manufacturing

Under current FDA EA Guidance (FDA, 1995), information concerning the introduction of substances into the environment from manufacturing information need not be included for foreign facilities with appropriate certification statements; therefore, manufacturing emissions and controls information is provided for U.S. manufacturing facilities only. Please see Confidential Appendices H-1 and H-2 for the route of synthesis of the drug substance, and Confidential Appendix H-5 for a description and flow chart of the "chlorohydrin" manufacturing process.

Redacted 1

page(s) of trade secret and/or confidential commercial information

i. Synthesis of the Proprietary Intermediate _____ Syntex Chemicals, Incorporated, Boulder, Colorado

(1) Atmospheric Emissions

The only regulated compounds directly related to chlorohydrin manufacturing that are expected to be emitted to the atmosphere are volatile organic compounds (VOCs). For a list of the specific VOCs expected to be emitted, please see Confidential Appendix H-6.

(2) Wastewater

Small amounts of organic compounds, salts, and unreacted starting materials and intermediates will be sewered during the synthesis of ' . For a list of the specific chemicals expected to be emitted, please see Confidential Appendix H-7.

(3) Non-Aqueous Liquid Waste

A non-aqueous wastestream, mainly consisting of organic solvents, is a byproduct of the synthesis of . For a list of the substances, please see Confidential Appendix H-8.

(4) Solid Waste

Small quantities of various types of filter cartridges and personal protective equipment are used during the production process. This spent, contaminated equipment is sent to permitted hazardous waste facilities. Please see Confidential Appendix H-9 for the names and addresses of the hazardous waste disposal facilities currently approved by Syntex for use, their permitting authorities, permit numbers and permit expiration dates.

ii. Synthesis of the Drug Substance, Hoffmann-La Roche Ltd., Basle, Switzerland

The synthesis of the drug substance saquinavir base will be carried out at the facilities of Hoffmann-La Roche in Basel, Switzerland, under carefully controlled conditions. Hoffmann-La Roche certifies that the manufacture of saquinavir base will be carried out in compliance with all applicable environmental regulations (Appendix E).

iii. Manufacture of the Drug Product

The manufacture of the drug product, TRADE NAME™ (saquinavir) Soft Gelatin Capsules (200 mg) will be carried out under carefully controlled conditions. certifies that the manufacture of the drug product will be carried out in compliance with all applicable environmental regulations (Appendix E).

iv. Packaging and Labeling of the Drug Product, Building 59, Hoffmann-La Roche Incorporated, Nutley, New Jersey

(1) Atmospheric Emissions

No organic solvents are used in the packaging and labeling of TRADE NAME™ (saquinavir) Soft Gelatin Capsules (200 mg). The finished drug product will be shipped in bulk from so atmospheric emissions of the formulated product or excipients are not expected.

(2) Wastewater

Emissions to wastewater are not expected from the packaging and labeling operations.

(3) Solid Waste

Solid waste, including waste packaging, off-specification and returned expired drug product, drug product used for laboratory testing or cleaned from machinery, and protective

clothing worn by operators will be collected for incineration on-site. Please see Appendix C for permit information pertinent to this operation.

b. Controls Exercised

i. Synthesis of the Proprietary Intermediate Chemicals, Incorporated, Boulder, Colorado

(1) Atmospheric Emissions

Condensers are installed on individual pieces of equipment potentially capable of emitting greater than 15 lb./day of VOCs. One high vapor pressure VOC, n-butane, which is formed as a reaction by-product, is controlled through cryogenic condensation of vapors emitted from all equipment where it is present. Other VOCs are condensed and collected by this cryogenic system in quantities that allow the n-butane to remain soluble in waste solvent streams. In addition, process equipment vents are manifolded and ducted through wet scrubbers employing water or caustic/water scrubbing solutions, and then through activated carbon adsorption units prior to discharge to the atmosphere.

(2) Wastewater

Wastewater from manufacturing is monitored for pH, flammability, and total organic carbon. The pH of the wastewater is adjusted, if necessary, after which it passes through an on-site biological pretreatment system that reduces the concentration of oxygen-demanding material. Sludge from the primary clarifier of the pretreatment facility is sent to a permitted hazardous waste incinerator. Please see Confidential Appendix H-9 for the names and addresses of the hazardous

waste disposal facilities currently approved by Syntex for use, their permitting authorities, permit numbers and permit expiration dates. The pretreated wastewater then passes to the City of Boulder publicly owned treatment works. The pretreatment facility operates under a pretreatment permit issued by the City of Boulder (Appendix F).

(3) Non-Aqueous Liquid Waste

The non-aqueous liquid wastes resulting from the synthesis of are drummed and transported by contractors to State-permitted hazardous waste facilities for disposition. . Please see Confidential Appendix H-9 for the names and addresses of the hazardous waste disposal facilities currently approved by Syntex for use, their permitting authorities, permit numbers and permit expiration dates.

(4) Solid Waste

Small quantities of various types of filter cartridges and personal protective equipment are used during the production process. This spent, contaminated equipment is sent to permitted hazardous waste facilities. Please see Confidential Appendix H-9 for the names and addresses of the hazardous waste disposal facilities currently approved by Syntex for use, their permitting authorities, permit numbers and permit expiration dates.

ii. Packaging and Labeling of the Drug Product, Building 59, Hoffmann-La Roche Incorporated, Nutley, New Jersey

(1) Atmospheric Emissions

Since atmospheric emissions of the formulated product or excipients are not expected from the packaging and labeling operations, no permits or controls are necessary.

(2) Wastewater

Since emissions to wastewater are not expected from the packaging and labeling operations, no permits or controls are necessary.

(3) Solid Waste

Solid waste, including waste packaging, off-specification and returned expired drug product, drug product used for laboratory testing or cleaned from machinery, and protective clothing worn by operators will be collected for incineration on-site. Please see Appendix C for permit information pertinent to this operation.

c. Citation of and Statement of Compliance with Applicable Emission Requirements

i. Synthesis of the Proprietary Intermediate Syntex Chemicals, Incorporated, Boulder, Colorado

In all manufacturing at its Boulder, Colorado facility, Syntex Chemicals, Incorporated is obliged to comply with the requirements stated in the following laws and regulations:

(1) Atmospheric Emissions

Federal Clean Air Act Amendments of 1990--Regulation of volatile organic compounds (VOCs) and hazardous air pollutants (HAPs)

Colorado Air Quality Control Act

Industrial and Commercial Users of Ozone-Depleting Substances, City of Boulder Ordinance

(2) Wastewater

Federal Water Pollution Control Act of 1972

Water Quality Act of 1987 as amended

Effluent Guidelines and Standards for Pharmaceuticals Manufacturing

Colorado Water Quality Control Act

Industrial and Prohibited Wastewater Discharges, City of Boulder Ordinance

Storm Water and Flood Management Utility, City of Boulder Ordinance

(3) Occupational

U.S. Occupational Safety and Health Act

(4) Solid/Hazardous Waste Transportation and Disposal

Resource Conservation and Recovery Act

Hazardous Material Transportation Act

Hazardous Material Transportation Uniform Safety Act

Emergency Planning and Community Right-to-Know Act

Toxic Substance Control Act

Colorado Hazardous Waste Act

Colorado Hazardous Waste Clean Up Act

Colorado Hazardous Waste Substance Incidents Law

Colorado Solid and Hazardous Waste Disposal Sites and Facilities Law

Pollution Prevention

Underground Storage Tanks

Solid Waste Disposal Sites and Facilities

City of Boulder Hazardous Material Transportation Ordinance

Syntex Chemicals, Incorporated certifies that it is in compliance with, or on an enforceable schedule to be in compliance with all emission requirements set forth in permits, consent decrees and administrative orders applicable to the synthesis of at its facilities in Boulder, Colorado (Appendix E).

For a list of the current permits applicable to emissions and wastes generated as a result of the synthesis of please see Appendix F.

ii. Packaging and Labeling of the Drug Product, Building 59, Hoffmann-La Roche Incorporated, Nutley, New Jersey

In all manufacturing at its Nutley, New Jersey facility, Hoffmann-La Roche Incorporated is obliged to comply with the requirements stated in the following laws and regulations:

(1) Atmospheric Emissions

Federal Clean Air Act Amendments of 1990--Regulation of volatile organic compounds (VOCs) and hazardous air pollutants (HAPs)

New Jersey Administrative Code (N.J.A.C.):

N.J.A.C. 7:27-6	Control and Prohibition of Particles from Manufacturing Process
N.J.A.C. 7:27-8	Permits and Certificates
N.J.A.C. 7:27-16	Control and Prohibition of Air Pollution by VOCs
N.J.A.C. 7:27-17	Control and Prohibition of Air Pollution by Toxic Substances
N.J.A.C. 7:27-19	Control and Prohibition of Air Pollution from Oxides of Nitrogen
N.J.A.C. 7:27-21	Emission Statements
N.J.A.C. 7:27-22	Operating Permits

(2) Wastewater

Federal Water Pollution Control Act of 1972

Water Quality Act of 1987 as amended

Effluent Guidelines and Standards for Pharmaceuticals Manufacturing

(3) Occupational

U.S. Occupational Safety and Health Act

New Jersey Toxic Catastrophe Prevention Act

New Jersey Worker and Community Right-To-Know Law

New Jersey Department of Health Regulations

(4) Solid/Hazardous Waste Transportation and Disposal

Resource Conservation and Recovery Act

New Jersey Solid Waste Management Act

Hazardous Material Transportation Act

Hazardous Material Transportation Uniform Safety Act

Hoffmann-La Roche Incorporated certifies that it is in compliance with, or on an enforceable schedule to be in compliance with all emission requirements set forth in permits, consent decrees and administrative orders applicable to the packaging and labeling of TRADE NAME™ (saquinavir) Soft Gelatin Capsules (200 mg) at its facilities in Nutley, New Jersey (Appendix E).

For a list of the current Hoffmann-La Roche permits applicable to the manufacture of TRADE NAME™ (saquinavir) Soft Gelatin Capsules (200 mg) (on-site incineration of solid non-hazardous waste), including permit numbers, issuing authorities, and expiration dates please see Appendix C.

d. Discussion of the Effect of Approval on Compliance with Current Emission Requirements

The current production estimate for the fifth year (2001) following approval of the NDA for which this document is applicable (Confidential Appendix H-3) indicates that manufacturing will not require significant expansion of facilities. The process will be scheduled to fit within the existing framework of activities for which current emission requirements are applicable. The packaging and labeling operations at Hoffmann-La Roche in Nutley, New Jersey will require no new equipment, and the proposed increase in the production c at the Syntex Chemicals, Inc., facility will not impact environmental compliance at the facility.

e. Expected Introduction Concentrations

i. Expected Introduction Concentration from Use

Saquinavir bioavailability is impacted by incomplete absorption and extensive first-pass metabolism. At least seven major human metabolites are excreted, predominantly via the biliary route. No single metabolite is expected to account for greater than 10% of the total of excreted drug-related substances.

To conservatively (i.e., maximally) estimate the amount of saquinavir and its metabolites eliminated by patients to a typical wastewater treatment plant, it is assumed that the production forecast for the U.S. in Confidential Appendix H-3 will equal the amount ingested and eliminated by the U.S. population.

Based on the estimated total U.S. production of saquinavir for use in both the hard and soft gelatin capsule dosage forms during the fifth year (2001) following approval of NDA No. 20-828, (Confidential Appendix H-3), an Expected Introduction Concentration (EIC) from use (FDA, 1995) of less than 10 µg of saquinavir and its metabolites per

liter of wastewater (parts per billion) at a typical wastewater treatment plant can be estimated. The calculations of this EIC are given in Confidential Appendix H-10.

ii. Expected Introduction Concentration from Disposal

Saquinavir will not enter the U.S. environment through emissions during manufacturing, because materials containing the drug substance will not be released during the packaging and labeling operations. Solid waste, including waste packaging, off-specification and returned expired drug product, drug product used for laboratory testing or cleaned from machinery, and protective clothing worn by operators will be collected for incineration on-site. A small amount of chlorohydrin will potentially be emitted to wastewater as a result of the synthesis operation at Syntex. The potential for environmental impacts resulting from this release has been considered by the appropriate Federal and State agencies in licensing and permitting processes; controls specified by these agencies have been instituted to ensure that the emissions are environmentally acceptable.

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

a. Identification of Substances of Interest

The pharmacokinetics of saquinavir can be characterized by limited bioavailability due to incomplete absorption and extensive first-pass metabolism. Biliary excretion of drug-related material accounts for the vast majority of the dose following oral administration. A metabolic profile of saquinavir in human urine and feces has not been determined, but the *in vitro* liver microsomal metabolic profile in man is qualitatively similar to that of other animal species studied, with at least seven major metabolites detected. Most of these metabolites arise via hydroxylation of the decahydroisoquinoline ring. One metabolite is apparently a product of oxidation of one of the methyl groups

of the tertiary butyl group to a carboxylic acid. The results of the microsomal study suggest that no one metabolite is likely to exceed 10% of the total of excreted drug-related substances. These metabolites are partially oxidized, and more soluble and more polar than the parent drug substance; therefore, they are more likely than the parent drug to be affected by environmental depletion processes, including photolysis, further oxidation, hydrolysis, and biodegradation. For this reason, this section will focus on the assessment of the fate and transport of any parent drug potentially emitted to the environment intact as a result of patient use.

Any saquinavir potentially excreted to wastewater from patients may be affected by environmental processes that could include photolysis, oxidation, hydrolysis, volatilization, adsorption, bioaccumulation, and biodegradation. These processes may occur during wastewater treatment or potentially in the surface water receiving the effluent from wastewater treatment facilities. Each process is evaluated in this section before a concluding statement is made regarding the probable fate and concentration of released substances in the aqueous environment.

b. Physical-Chemical Characterization

For a physical-chemical characterization of the drug substance, please see Table 5-1 on page 8.

i. Water Solubility

Saquinavir is only sparingly soluble in water, indicating that even in a worst-case scenario involving a spill of the drug substance, concentrations in the aquatic compartment would be limited to 3 mg/L (parts per million)(a saturated solution) or less.

ii. Dissociation Constant

The only environmentally relevant dissociation constant of saquinavir base, that of 6.89 ± 0.02 for the proton of the

decahydroisoquinoline group, indicates that saquinavir base would exist in the aquatic compartment in an equilibrium consisting of roughly equal concentrations of molecules containing either protonated or unprotonated decahydroisoquinoline groups.

The mesylate salt of saquinavir has a dissociation constant for the proton of the decahydroisoquinoline group of 7.1 ± 0.2, as reported in the EA for NDA No. 20-~~682~~ 628. As expected, the confidence intervals for the estimates of the dissociation constants for the decahydroisoquinoline proton for the mesylate salt and the free base overlap, indicating that they are not significantly different from each other. Based on the lack of difference in these dissociation constants, it can be concluded that once the mesylate salt of saquinavir is dissolved in water and dissociates into the free base and the methylsulfonic acid, the ratio of protonated to unprotonated decahydroisoquinoline groups in the saquinavir free base solution is the same irrespective of which form of the drug substance was used initially to create that solution. Therefore, the aquatic fate and effects studies performed with the mesylate salt of the drug substance to support the EA for NDA No. 20-~~682~~ 628 are equally applicable in support of the EA for this submission, because the same molecular structures were actually the subject of the studies.

iii. Octanol/Water Partition Coefficient

At the environmentally relevant pH of 7.4, the log P of saquinavir base is 3.34. This value indicates that the drug substance will exhibit a tendency to adsorb to the organic fraction of soil, sediment or sludge, is somewhat lipophilic, and may exhibit a potential to bioaccumulate. However, since the drug substance is extensively metabolized, very little of the intact drug substance is expected to be

available in the aquatic compartment to become adsorbed to solid organic substrata in the environment or to bioaccumulate in organisms. Saquinavir may be bound so strongly to the organic carbon in sediment that it is no longer bioavailable. Most adsorption of any intact drug substance present in wastewater would be expected to occur during wastewater treatment as a result of contact with organic carbon in activated sewage sludge solids. In the U.S., most of this material is typically subsequently incinerated or landfilled, along with any adsorbed saquinavir, removing any adsorbed drug substance from the environment.

The drug substance has a relatively high molecular weight, which tends to impede its passage through biological membranes. At a pH of 7.4, the Bioconcentration Factor (BCF) can be estimated from the octanol/water partition coefficient to be approximately 203 (Lyman *et al.*, 1982). For this calculation, please see Confidential Appendix H-11). This value compares favorably to the BCF values of chemicals known to bioaccumulate, such as DDT (BCF = 61,600) and Arochlor (BCF = 100,000). For purposes of comparison, carbon tetrachloride, which has not been implicated in bioaccumulation processes, has a BCF of 30. Therefore, based on the BCF and the extremely low Expected Environmental Concentration, significant bioaccumulation of saquinavir in aquatic organisms is not expected.

iv. Vapor Pressure or Henry's Law Constant

The vapor pressure of a substance is a property which influences the tendency of that substance to volatize from water to air, and to be transported in the atmosphere. The property is an important parameter to consider in predicting the distribution of chemicals into the various environmental compartments. Based on the molecular weight of the

drug substance, 670.855, and the structure, the vapor pressure can be estimated to be less than 1 x 10^{-7} torr. This low vapor pressure, and the strong tendency of saquinavir to adsorb to organic carbon in soil (Confidential Appendix H-12), indicates a negligible potential for saquinavir to partition to the atmospheric compartment of the environment.

c. Environmental Depletion Mechanisms

An aerobic biodegradation study was performed to determine whether saquinavir would undergo significant biodegradation during wastewater treatment (Confidential Appendix H-13). The results indicated that saquinavir was not biodegradable under the conditions of the study. This does not mean that the drug substance is not susceptible to biodegradation during wastewater treatment or in the natural environment, however, as it may be biodegraded if it is exposed to enzymes induced for biodegradation of other substances. Furthermore, saquinavir would probably be biotransformed via the wastewater treatment process even though complete mineralization was not evidenced to a significant extent during the biodegradation study.

The UV-visible spectrophotometric absorption spectrum of saquinavir exhibits no significant absorption within the wavelength range of terrestrial sunlight (Table 5-1). Therefore, photodegradation in surface water is not expected.

Based on the structure of saquinavir and the results of drug substance stability studies performed during development, significant uncatalyzed, abiotic hydrolysis is not expected to occur under environmental conditions. However, the hydrolysis of saquinavir may potentially occur in the aquatic environment via catalysis by extracellular enzymes. Fungi secrete extracellular enzymes to break down molecules that are either immobile or too large for transport into the cell. An example of this phenomenon is the degradation of lignin by

extracellular hydrolases and oxidases. The non-specific nature of this catalysis with regard to substrata permits fungi to decompose a wide variety of synthetic chemicals in the environment. Hydrolysis of saquinavir via such catalysis would require the presence of fungi and naturally occurring macromolecules to induce secretion of the fungal enzymes.

Photochemically produced hydroxyl radicals in water have been observed to oxidize many organic chemicals, including nitrogen-containing heterocyclics. The hydroxyl radicals are generated in surface water from the photolysis of naturally occurring substances that absorb terrestrial sunlight. From the rate constants for these oxidations and from observations on the concentrations of hydroxyl radicals in typical surface waters, the half-life for degradation of most organic substances by this pathway has been calculated to be about 80 days.

Based on the results of the soil adsorption/desorption study performed with saquinavir mesylate (Confidential Appendix H-12), the drug substance can be expected to adsorb strongly to activated sludge solids. This depletion mechanism will significantly lower the concentration of any saquinavir potentially present in wastewater treatment plant effluent.

d. Expected Environmental Concentration (EEC)

The EEC is the expected concentration of saquinavir base that organisms would be exposed to in the environment after consideration of depletion factors. The EEC of saquinavir will be significantly lower than the Expected Introduction Concentration (EIC) in wastewater prior to treatment, due to dilution in the aquatic environment and depletion as a result of adsorption to organic carbon in activated sludge solids during wastewater treatment.

The EIC of saquinavir and its metabolites in wastewater is less than 10 µg/L (parts per billion). The effluent from a wastewater treatment plant outfall

is typically diluted extensively in the mixing zone of the receiving stream. Dilution factors can range from 0 in discharges to intermittent streams to many thousands in discharges to large rivers, but an effluent to receiving water body dilution factor of 0.0234 for a typical wastewater treatment facility/receiving water body scenario can be calculated from the volume of effluent entering publicly-owned treatment works, 1.115×10^{11} L/d (FDA, 1995), and the U.S. average daily runoff volume of 4.654×10^{12} L/d (Mays, 1996). Assuming that no other depletion mechanisms mitigate the concentration of saquinavir and its metabolites present in the aquatic environment, the concentration of these substances in completely mixed surface water downstream from a typical wastewater treatment plant outfall would average less than 0.3 μg/L (part per billion), on the basis of dilution alone.

As discussed above, saquinavir readily adsorbs to organic carbon in soil, indicating that adsorption to organic carbon in activated sludge solids will significantly deplete the concentration of saquinavir in the aquatic environment. The organic carbon content of the soil is probably the most important property determining the sorption of nonionic chemicals; since activated sewage sludge contains approximately 10 times more organic carbon than the soils used in the soil adsorption/desorption studies performed with saquinavir (Confidential Appendix H-12), the soil study results probably underestimate the capacity of activated sludge solids to bind saquinavir. The significance of the depletion of saquinavir potentially present in wastewater depends on the kinetics of the adsorption to activated sludge solids, relative to the typical wastewater residence time during treatment, as well as the magnitude of the adsorption. The soil adsorption study results indicated that the adsorption took place rapidly, with more than 90% of the saquinavir in the water phase adsorbed to the three soil types used within 48 hours. Since the contact time of wastewater in the activated sludge basin of a typical wastewater treatment plant is 4-8 hours

(up to 24 hours in extended aeration facilities), depletion due to adsorption to the organic carbon in activated sludge solids is expected to significantly lower the concentration of any saquinavir potentially passing through wastewater treatment plants, perhaps by as much as an order of magnitude. However, to maximally (conservatively) estimate the EEC, it will be assumed that the concentration of saquinavir in wastewater is depleted by 50% due to adsorption to organic carbon in activated sludge, thereby reducing the EEC to less than 0.15 µg/L (part per billion).

e. Summary

i. Aquatic Compartment

Excreted saquinavir and its metabolites are expected to enter the aquatic compartment via wastewater. No single metabolite or the parent drug is expected to account for more than 10% of the total amount of drug-related material in wastewater. Dilution and dissipation in receiving waters are expected to reduce the environmental concentrations of these substances by at least an order of magnitude upon mixing. Adsorption to activated sludge during wastewater treatment is expected to be an environmentally important depletion mechanism, significantly decreasing the concentration of any saquinavir potentially present in effluent from wastewater treatment facilities.

ii. Terrestrial Compartment

If saquinavir is released intact to wastewater, a significant portion will be adsorbed to organic carbon in sewage sludge solids. If saquinavir is present in sewage sludge solids subsequently applied to land, then a potential exists for saquinavir to enter the terrestrial environment. This point will be discussed in Section 8. However, most sewage sludge is digested, which may biotransform or mineralize any saquinavir adsorbed to it. Digested or dewatered sludge is typically

either incinerated or landfilled. If the sludge is incinerated, any saquinavir adsorbed to it would be destroyed. If the sludge is landfilled, any saquinavir adsorbed to it would no longer be bioavailable. Saquinavir may be so tightly bound to the organic carbon in sewage sludge solids that its bioavailability is negligible. Based on the bioconcentration factor of saquinavir, estimated from the octanol/water partition coefficient, significant bioaccumulation in soil-dwelling organisms is not expected.

iii. Atmospheric Compartment

Due to the low vapor pressure of saquinavir and its tendency to adsorb to organic carbon in soils and activated sludge, it is not expected to enter the atmosphere as a result of use.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

a. Aquatic Compartment

Five aquatic environmental effects studies and one terrestrial effects study have been conducted with saquinavir mesylate: (1) *Daphnia* acute toxicity (Confidential Appendix H-14), (2) Rainbow trout acute toxicity (Confidential Appendix H-15), (3) microbial growth inhibition (Confidential Appendix H-16), (4) green algal growth inhibition (Confidential Appendix H-17), (5) activated sludge respiration inhibition (Confidential Appendix H-18), and (6) earthworm sub-acute toxicity (Confidential Appendix H-19). All of the studies supporting this EA were conducted under FDA Good Laboratory Practice Guidelines (GLPs). All of the studies supporting this EA were conducted in accordance with the guidelines in FDA's Environmental Assessment Technical Assistance Handbook (1987), with the exception of the activated sludge respiration study, which was conducted under the european Organization for Economic Cooperation and Development (OECD) Guidelines. The results are presented in Table 8-1.

Table 8-1

Aquatic and Terrestrial Effects Study Results

Study and Species	No Observed Effect Concentration (mg/L)	LC_{50}, EC_{50}, or Minimum Inhibitory Concentration, mg/L
Acute Toxicity Study: *Daphnia magna*	36	$EC_{50} > 100$ (immobilization)
Acute Toxicity Study: *Oncorhynchus mykiss*	38	$LC_{50} > 38$
Microbial Inhibition Study:		
Azotobacter vinelandii		MIC > 312
Pseudomonas putida		MIC > 312
Anabaena flos-aquae		MIC = 312
Fusarium acuminatum		MIC > 312
Aspergillus niger		MIC > 312
Activated Sludge Respiration Inhibition Study		MIC > 58.8
Algal Growth Inhibition Study: (*Selenastrum capricornutum*)	10.4 (growth)	$EC_{50} = 20.5$ (biomass)
Earthworm Subacute Toxicity Study: (*Lumbricus terrestris*)	686 mg/kg soil	$LC_{50} > 686$

The lowest No Observed Effect Concentration (NOEC) for all tested organisms is the value of 10.4 mg/L for green algal growth. The next higher NOEC is 36 mg/L for the immobilization of water fleas. Of the five microbial organisms tested, a Minimum Inhibitory Concentration (MIC) could be determined for only one--the cyanobacterium (blue-green alga) *Anabaena flos-aquae*. The MIC for this organism was 312 mg/L.

In Section 7, the concentration of saquinavir and its metabolites in a typical water body receiving wastewater treatment plant effluent was estimated to be less than 0.15 µg/L (parts per billion), based on the effects of dilution and adsorption to the organic carbon in activated sewage sludge as depletion processes that will mitigate the concentration of less than 10 µg/L in wastewater prior to treatment. The estimated concentration of saquinavir and its metabolites in receiving waters is close to six orders of magnitude lower than the lowest no observed effect concentration determined in the aquatic effects studies. Although it is obvious that the concentrations of saquinavir in the environment that could potentially result from approval of the proposed action will not have an adverse effect on aquatic organisms, an additional margin of safety is provided because although the toxicity studies were performed using saquinavir drug substance as the test article, organisms in the environment will be exposed to a complex mixture of metabolites and degradation products, which will likely be less toxic than saquinavir, based on their increased polarity and water solubility. Moreover, it must be pointed out that other potential depletion mechanisms, including hydrolysis via extracellular enzymes secreted by fungi and oxidation via hydroxy radicals in surface waters may be important depletion mechanisms that have not been considered in the calculation of the EEC.

b. Terrestrial Compartment

Saquinavir will adsorb to activated sludge that may potentially be exposed to the drug substance, and this sludge may then subsequently serve as an exposure route for terrestrial organisms, as a result of sludge disposal by land application. The potential for effects on soil-dwelling organisms was evaluated by conducting an earthworm subacute toxicity study with saquinavir mesylate (Confidential Appendix H-19). The results, an NOEC of 686 mg/kg soil (parts per million), showed no toxicity to earthworms at the highest tested concentration.

A worst-case expected concentration of less than 36 mg/kg activated sludge can be estimated by calculating the amount of saquinavir expected to sorb to organic carbon in activated sludge, based on the K_d determined in various soils as a function of organic carbon content, and the proportion of activated sludge consisting of organic carbon (Lyman *et al.*, 1982). Please see Confidential Appendix H-20 for this calculation. During operations involving the application of sewage sludge to land, a sludge application rate of up to 3,000 lb./acre is typical (Water Pollution Control Federation, 1976), and the weight average of an acre of soil to a depth of 6-7 inches is approximately 2.5 million pounds (Brady). The dilution factor provided by mixing the sludge with soil at this ratio is approximately 0.0012, so a reasonable estimate of the EEC for saquinavir in soil resulting from the land application of activated sludge is less than 43 µg/kg soil (parts per billion). It must be pointed out that this value ignores any soil depletion mechanisms, such as photolysis or biodegradation, potentially capable of mitigating the saquinavir soil concentration calculated.

The NOEC of saquinavir to earthworms is more than four orders of magnitude greater than the worst case concentration expected in soil, indicating that the concentrations in soil that could potentially result from approval of the proposed action will not have an adverse effect on soil-dwelling organisms.

c. Effects on Wastewater Treatment Plants

Based on the EIC of less than 10 µg/L (parts per billion), and the results of the activated sludge respiration inhibition study (Confidential Appendix H-18), in which a Minimum Inhibitory Concentration (MIC) of greater than 58.8 parts per million for inhibition of activated sludge respiration was determined, saquinavir and its metabolites are not expected to affect wastewater treatment facilities.

9. USE OF RESOURCES AND ENERGY

a. Natural Resources and Energy

The proposed action does not require a large commitment of resources. With the exception of the proprietary intermediate, "chlorohydrin," all other chemicals used in the synthesis are commercially available commodities. "Chlorohydrin" is manufactured at Syntex with existing equipment, and the increase in production as a result of the proposed action is not expected to impact environmental compliance. The packaging and labeling operation at Hoffmann-La Roche Incorporated in Nutley, New Jersey will require no new equipment. Moreover, no irreversible or irretrievable commitment of limited national resources will be involved. With respect to the packaging and labeling operations at Hoffmann-La Roche in Nutley, New Jersey, the new dosage form of the drug product is expected to replace the older hard gelatin capsule formulation, resulting in a decrease in the total energy requirement for Building 59, relevant to the combined manufacture of both dosage forms containing saquinavir, due to the decrease in the volume of hard gelatin capsules manufactured there following approval of the improved dosage form. The soft gelatin capsules are manufactured abroad and shipped to Hoffmann-La Roche in bulk.

b. Effect on Endangered or Threatened Species

The facilities used for the production of saquinavir and TRADE NAME™ (saquinavir) Soft Gelatin Capsules (200 mg) are already committed to the production of pharmaceuticals, vitamins, and fine chemicals. No effects are expected on endangered or threatened species as a result of the proposed action.

c. Effect on Property Listed in or Eligible for Listing in the National Register of Historic Places

There are no effects expected upon property listed in or eligible for listing in the National Register of Historic Places as a result of the proposed action.

10. MITIGATION MEASURES

Environmental impacts associated with the production of saquinavir and the packaging and labeling of TRADE NAME™ (saquinavir) Soft Gelatin Capsules will be avoided or mitigated by the use of appropriate control measures in accord with all federal, state, and local regulations. Air emissions control devices include vent condensers, scrubbers and fabric filter dust collectors. Environmental impacts associated with the disposition of drug substance and/or metabolites following consumption by patients will be mitigated by conventional wastewater treatment plants. All rejected or expired TRADE NAME™ (saquinavir) Soft Gelatin Capsules will be incinerated on-site as nonhazardous solid waste.

Waste minimization is considered in the design of Hoffmann-La Roche processes to the extent possible while maintaining the quality and purity of the manufactured products. Solvents are recovered and reused within the same process or for different processes depending on quality control specifications. Yield maximization is an important consideration at all stages of process development.

The Hoffmann-La Roche facility in Nutley New Jersey is classified as a major facility under Chapter 7:1E of the New Jersey Administrative Code covering discharge

of petroleum and other hazardous substances. As a result, the facility is required to maintain plans for discharge prevention, containment and countermeasures, and discharge, cleanup and removal acceptable to the New Jersey Department of Environmental Protection. In addition to the physical facilities for the containment of spills, three emergency squads have been established at the Nutley site, consisting of over 90 volunteers from various departments, who respond to emergencies and actively participate in training and drills with local emergency planning committees.

The Roche Environmental Response Squad is a group of highly trained professionals who respond immediately and effectively to potential spills of hazardous substances at the Nutley site. It consists of individuals with a balanced set of technical expertise and skills in such plant activities as the tank farm, boiler operations, wastewater treatment, chemical processing, warehousing, and laboratory operations. The Environmental Response Squad works closely with the other emergency squads and with the Chemical Production Department personnel in order to coordinate effective and efficient responses to potential environmental emergency situations.

11. ALTERNATIVES TO THE PROPOSED ACTION

Alternatives available to the FDA in response to this proposed action include approval of the proposed action through the issuance of a Finding of No Significant Impact and non-approval and notification of intent to prepare an Environmental Impact Statement.

We believe that the first alternative, issuance of a Finding of No Significant Impact, is fully justified by this Environmental Assessment. Manufacturing operations will be carried out in compliance with the regulations of all applicable governmental regulatory authorities. Releases of saquinavir to the environment will be mitigated as discussed in Sections 6 and 10. Fate and effects studies were carried out and interpreted in Sections 7 and 8, and support the position that the use of TRADE NAME™ (saquinavir) Soft Gelatin Capsules (200 mg) will not adversely impact the environment.

12. PREPARER

This Environmental Assessment was prepared by Stanley Rodgers, Senior Corporate Environmental, Health and Safety Professional, Corporate Environmental and Safety Affairs, Hoffmann-La Roche Incorporated. Mr. Rodgers previously managed the Environmental Assessment function for Sandoz Pharmaceuticals Corporation, and is an ecotoxicologist formerly employed by Exxon Biomedical Sciences, Inc., where he supervised laboratory operations and directed GLP compliant environmental effects studies to support registration of chemicals under FIFRA, TSCA, OECD, and FDA guidelines. He holds an M.S. degree in Biology with minors in Chemistry and Statistics.

13. CERTIFICATION

The undersigned official certifies that the information presented in this Environmental Assessment is true, accurate, and complete to the best of the knowledge of the persons responsible for preparation of the Environmental Assessment.

The undersigned official certifies that the information presented under items 1 through 15 of the Environmental Assessment and Appendices A-G contain non-confidential information and acknowledges that this information will be made available to the public in accordance with 40 CFR Part 1506.6.

Signature Jack S Kace Date 4/29/97

Jack S. Kace, Eng. Sc.D
Vice President and Director
Corporate Environmental and Safety Affairs
Hoffmann-La Roche Incorporated

14. REFERENCES

Brady, N.C., The Nature and Properties of Soils, 8th Edition, New York: Macmillan Publishing Co., Inc.

Mays, L.W., ed., 1996. Water Resources Handbook, New York: McGraw Hill Book Company.

Lyman, W.J., Rheel, W.F., Rosenblat, D.H., eds., 1982. Handbook of Chemical Property Estimation Methods, New York: McGraw Hill Book Company.

U.S. Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 1995. Guidance for Industry--for the Submission of an Environmental Assessment in Human Drug Applications and Supplements.

Water Pollution Control Federation, 1976. Operation of Wastewater Treatment Plants, Lancaster, Pennsylvania. Lancaster Press.

15. APPENDICES

A: DOCUMENTATION OF THE FDA AGREEMENT THAT "
WILL BE DEFINED AS STARTING MATERIALS

B: ENVIRONMENTAL SETTINGS OF U.S. MANUFACTURING FACILITIES

C: HOFFMANN-LA ROCHE EMISSIONS PERMIT INFORMATION

D: MATERIAL SAFETY DATA SHEET FOR SAQUINAVIR MESYLATE

E: SELF CERTIFICATIONS OF ENVIRONMENTAL COMPLIANCE

F: SYNTEX CHEMICALS, INCORPORATED EMISSIONS PERMIT INFORMATION

H-13 AEROBIC BIODEGRADATION OF SAQUINAVIR MESYLATE IN WATER

H-14 ACUTE TOXICITY OF SAQUINAVIR MESYLATE TO *DAPHNIA MAGNA*

H-15: ACUTE TOXICITY OF SAQUINAVIR MESYLATE TO RAINBOW TROUT

H-16 MICROBIAL GROWTH INHIBITION OF SAQUINAVIR MESYLATE

H-17 GROWTH INHIBITION OF SAQUINAVIR MESYLATE TO GREEN ALGAE

H-18: ACTIVATED SLUDGE RESPIRATION INHIBITION STUDY WITH SAQUINAVIR MESYLATE

H-19: EARTHWORM SUB-ACUTE TOXICITY STUDY WITH SAQUINAVIR MESYLATE

H-20: CALCULATION OF THE CONCENTRATION OF SAQUINAVIR IN ACTIVATED SEWAGE SLUDGE

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confidential

commercial

information

APPENDIX B

ENVIRONMENTAL SETTINGS OF U.S. MANUFACTURING FACILITIES

Environmental Settings of U.S. Manufacturing Facilities

Hoffmann-La Roche Incorporated, Nutley, New Jersey:

The Hoffmann-La Roche Incorporated facility is located approximately 10 miles west of New York City in Nutley, New Jersey. The Nutley facility is located in an industrial/residential area. State Route 3 runs along the north boundary of the site. The Passaic River is located approximately one mile east of the plant. The facility occupies approximately 122 acres of land and is mostly occupied by office, research and manufacturing buildings. The entire state of New Jersey is a non-attainment zone for ozone. The Nutley environs are in attainment for all other criteria air pollutants. The Roche Nutley facility is a manufacturing site for pharmaceuticals and chemicals for the Roche Group. It is also a research and an administrative site (U.S. Headquarters) for the Roche Group.

Syntex Chemicals, Incorporated, Boulder, Colorado:

The Syntex facility is located within the city limits of Boulder, Colorado, along the foothills of the Colorado Rocky Mountains. The immediate area is primarily commercial with a few adjacent industrial facilities. The climate is temperate with short, intense periods of snowfall in the fall and winter and short, intense periods of thundershowers in the spring and summer.

APPENDIX C

HOFFMANN-LA ROCHE EMISSIONS

PERMIT INFORMATION

Hoffmann-La Roche Incorporated Emissions Permit Information:

Unless otherwise noted, the permitting authority is the New Jersey Department of Environmental Protection.

Air:

No emissions to the atmosphere are anticipated for the packaging and labeling operation at Hoffmann-La Roche, so no permits are required.

Wastewater:

No emissions to wastewater are anticipated for the packaging and labeling operation at Hoffmann-La Roche, so no permits are required.

Solid Waste:

The air permit number for the on-site medical waste (solid non-hazardous waste) incinerator is 113190. The conditional permit to operate emissions control equipment is automatically renewed every 90 days.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

APPENDIX D

MATERIAL SAFETY DATA SHEET

FOR SAQUINAVIR MESYLATE

Material Name: Saquinavir mesylate
Material Code: 50536
MSDS Number .: m-003287.asc

Approved: 08/01/94

ROCHE LABORATORIES
a division of Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, NJ 07110-1199

Emergency: (201) 235-6660
Chemtrec: (800)-424-9300
Information: (800) 526-6367

MATERIAL SAFETY DATA SHEET

SECTION 1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Material Name .....: Saquinavir mesylate
Inventory Code .....: 50536
RO # .....: 31-8959/003
Synonyms .....: N-tert-Butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide methanesulfonate
TSCA Status .....: FDA Exemption - Not on Inventory; R&D Exemption - Not on Inventory.
Chemical Family ....: Peptide-mimetic class
Therapeutic Category: Antiviral agent (HIV proteinase inhibitor)
Formulations Used In: INVIRASE(TM)

SECTION 2. COMPOSITION/INFORMATION ON INGREDIENTS

Ingredient Name	CAS Number	Concentration %
Saquinavir mesylate		>=98.5

SECTION 3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW
Physical State .....: Fine Powder.
Color .....: White to off-white

Severe dust explosion hazard.

POTENTIAL HEALTH EFFECTS
Relevant Routes of Exposure .....: Inhalation, Ingestion.
Target Organs .....: Central Nervous System.

Acute Effects
General .....: May cause central nervous system effects such as headache, dizziness, drowsiness, fatigue, and lack of muscular coordination.

Chronic Effects ....: No adverse effects known.

Carcinogenicity ....: Not listed by NTP, IARC, or OSHA.

SECTION 4. FIRST AID MEASURES

Inhalation .....: Remove to fresh air. If discomfort occurs or persists, get medical attention.

Skin Contact .....: Remove contaminated clothing and shoes. Flush skin with plenty of water. If irritation occurs or persists, get medical attention. Wash clothing and shoes before reuse.

Eye Contact .....: Immediately flush eyes with plenty of water. If irritation occurs or persists, get medical attention.

Ingestion .....: If large quantities of this material are swallowed, get medical attention immediately. Do not induce vomiting unless directed by medical personnel. Never give anything by mouth to an unconscious person.

SECTION 5. FIRE FIGHTING MEASURES

Flash Point .....: Not Applicable

Extinguishing Media : Water, Carbon Dioxide, Dry Chemical, Foam.

Unusual Fire and Explosion Hazards ..: Severe dust explosion hazard. Toxic emissions may be given off in a fire. See Decomposition Products in Section 10. Stability and Reactivity.

Fire Fighting Instructions .....: Wear NIOSH/MSHA approved positive pressure, self contained breathing apparatus and full protective turn out gear. Use caution in approaching fire. Use water to keep fire exposed containers cool.

ST number .....: 2, Hartmann Tube.

SECTION 6. ACCIDENTAL RELEASE MEASURES

Spill Clean Up Procedures .....: Use proper personal protective equipment and clothing specified in Section 8-Exposure Controls/Personal Protection. Shut off the source of the spill or leak if it is safe to do so. Shut off all electrical equipment if it is safe to do so. Eliminate possible ignition sources. Follow appropriate grounding procedures. Scoop or shovel spilled material into a suitable labeled open head drum. Secure the drum cover and move the container to a safe holding area. Mop or flush the area with water. Collect wash with a noncombustible absorbent material and transfer to labeled container for treatment and disposal. Check area for residual material and repeat clean up if detected.

Treatment and Disposal .....: Decontaminate equipment. Dispose of protective clothing with the spilled material. Dispose of in accordance with recommendations in Section 13 Disposal Considerations.

SECTION 6. ACCIDENTAL RELEASE MEASURES (Continued. . .)

Reporting Requirements .....: The United States Environmental Protection Agency (USEPA) has not established a Reportable Quantity (RQ) for releases of this material. In New Jersey, report all releases which are likely to endanger the public health, harm the environment or cause a complaint to the NJDEPE Hotline (1-609-292-5560) and to local officials. State and local regulations vary and may impose additional reporting requirements.

SECTION 7. HANDLING AND STORAGE

Special Sensitivity : Heat. Do not heat above 170 degrees C.

Handling & Storage Precautions .....:
Do not generate dust or expose to ignition sources.
Ground and bond all transfer equipment.
Milling/mixing/drying should be performed in devices equipped with explosion relief or suppression systems or under inert conditions.
Avoid contact with eyes, skin and clothing.
Avoid breathing dust.
Use with adequate ventilation.
When handling, use proper personal protective equipment specified in section 8.
Wash thoroughly after handling.
Keep container tightly closed when not in use.
Store in a cool, dry area.

SECTION 8. EXPOSURE CONTROLS / PERSONAL PROTECTION

ENGINEERING CONTROLS
Ventilation .....: Local ventilation is recommended when using this material. Use in a lab hood.

PERSONAL PROTECTION
Respirator Type(s) .: Negative Pressure Air Purifying, Half Face, Toxic Dust/Mist/Fume High Efficiency Filter.

Conditions for Use .: Respiratory protection is recommended under excessively dusty conditions. For production operations, a supplied-air full facepiece respirator or supplied-air hood is recommended. OSHA considers effective engineering controls to be the primary means to control worker exposure. Respiratory protection should not substitute for feasible engineering controls. Whenever respiratory protection is used, a complete respirator program should be developed in accordance with OSHA Subpart I (29CFR1910.134) requirements.

`love Materials ....: Any plastic or rubber glove.

onditions for Use .: Gloves are required if there is a potential for skin

SECTION 8. EXPOSURE CONTROLS / PERSONAL PROTECTION (Continued. . .)

Skin Protection ....: Use protective clothing (disposable coveralls, lab coats, etc.) in both production and laboratory areas. Consult the protective clothing manufacturer, supplier and/or industrial hygienist.

Eye Protection .....: Safety Glasses Required.

OTHER CONTROL MEASURES

Administrative Controls .....: Post the work area and limit access to authorized personnel only.

Additional Protective Measures : Work clothing should be removed in a changeroom on site and laundered professionally. Employees should shower and change into street clothes before leaving the facility. Provide safety showers and eyewash stations in the work area. Prevent the accumulation of dust in the work area by thorough periodic cleaning of the area.

EXPOSURE LIMITS

There are no exposure limits specified either for this material or for any of its ingredients.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State ....: Fine Powder.
Color .....: White to off-white
Molecular Weight ...: 766.96
Chemical Formula ...: C38H50N6O5.CH4O3S (1:1)
Pure/Mixture .....: Pure.
Melting Point .....: 244 C
H2O Solubility .....: 2.20 g/l; Slightly Soluble (0.1-1% by weight).
Solubility - Other .: Ethanol

SECTION 10. STABILITY AND REACTIVITY

Stability .....: Normally stable but may become unstable at elevated temperatures or reacts with water, releasing some energy but not violently.

Conditions to Avoid : Temperatures >100C
Dust Accumulation
Airborne Dust
Sources of Ignition

Incompatibility - Materials to Avoid .: Unknown.

Decomposition Products .....: Carbon monoxide, carbon dioxide, oxides of nitrogen, oxides of sulfur

Decomposition

SECTION 10. STABILITY AND REACTIVITY (Continued. . .)

Conditions of
Polymerization .....: Will not occur.

SECTION 11. TOXICOLOGICAL INFORMATION

Saquinavir mesylate

Acute Oral, Single Dose, Rat: >5000 mg/kg
Summary: The oral LD50 is greater than 5,000 mg/kg body weight (limit test) under the study conditions utilized.

Irritation Skin, 4 hour, Rabbit
Summary: No skin irritation was observed in rabbits after being exposed for 4 hours to a 0.5 g aliquot of this material.

Mutagenicity
Summary: No evidence of mutagenicity was observed in the Ames assay with or without metabolic activation, the unscheduled DNA synthesis assay, the mouse micronucleus test, the mammalian cell gene mutation assay (V79/HGPRT) and the chromosomal aberration assay with or without metabolic activation.

Reproductive Oral, Rat
Summary: No adverse effects were observed in peri and post-natal studies in rats at oral doses up to 1,600 mg/kg/day under the study conditions utilized. Also, no adverse effects were observed in a fertility and general reproductive study in rats at oral doses up to 1,200 mg/kg/day under the study conditions utilized.

Teratogenicity Oral, Rabbit
Summary: No evidence of teratogenicity was observed in rabbits who were treated orally with doses up to 1,000 mg/kg/day during gestation days 7 through 18 under the study conditions utilized.

Teratogenicity Oral, Rat
Summary: No evidence of teratogenicity was observed in rats who were treated orally with doses up to 1,600 mg/kg/day during gestation days 6 through 15 under the study conditions utilized.

Sensitization Skin, Guinea Pig
Summary: No evidence of sensitization was observed in a Guinea Pig Maximization Test under the study conditions utilized.

SECTION 12. ECOLOGICAL INFORMATION

No ecological data available on this material.

SECTION 13. DISPOSAL CONSIDERATIONS

Disposal
Recommendations ....: This material is suitable for incineration. These recommendations are based on the product as shipped. Use, processing, alteration or contamination may affect these disposal recommendations. State, local or site restrictions affecting the available proper disposal options may vary.
RCRA Waste # .....: Not regulated under RCRA
Empty Containers ...: Empty containers must be triple rinsed prior to disposal, recycling, or reuse.

SECTION 14. TRANSPORTATION INFORMATION

Enforcement Agency .: US Dept. of Transportation
Country/Community ..: USA
Proper Ship. Name ..: Non-regulated

Enforcement Agency .: International Air Transport Association
Country/Community ..: International
Proper Ship. Name ..: Non-regulated

SECTION 15. REGULATORY INFORMATION

No regulatory information available on this material.

SECTION 16. OTHER INFORMATION

APPROVAL INFORMATION
Preparer .....: Annette Bucca-Janacek
Approver .....: Corporate Environmental & Safety Affairs
Approval Date .....: 08/01/94
Reason For Issue ...: New MSDS

The information presented on this MSDS is, to the best of our knowledge, accurate and reliable. It is provided in good faith without warranty or acceptance of any liability on the part of Hoffmann-LaRoche, Inc. It is the responsibility of the user to evaluate the relevance and completeness of this information for their application and to determine the safety, suitability and status under applicable regulations relating to this product or

APPENDIX E

SELF CERTIFICATIONS OF

ENVIRONMENTAL COMPLIANCE

Edward C. Thiele
Vice President, Technical Operations

Roche

April 2, 1997

SELF-CERTIFICATION OF ENVIRONMENTAL COMPLIANCE

To whom it may concern:

Hoffmann-La Roche Inc. states that it is in compliance with, or on a schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the packaging and labeling of Invirase® EOF™ (saquinavir) Capsules (200 mg) at its facilities in Nutley, New Jersey, as well as emission requirements set forth in federal, state, and local statutes and regulations applicable to the packaging and labeling of Invirase® EOF™ (saquinavir) Capsules (200 mg) at its facilities in Nutley, New Jersey. The approval and the subsequent increase in production is not expected to affect compliance with current emission requirements or compliance with environmental laws.

SYNTEX CHEMICALS, INC.
2075 NORTH 55TH STREET
BOULDER, COLORADO 80301-2880

(303) 442-1926
FAX: (303) 938-6413

SYNTEX

CERTIFICATION OF ENVIRONMENTAL COMPLIANCE
SYNTEX CHEMICALS, INC.
BOULDER, COLORADO

February 3, 1997

SIGNATORY CERTIFICATION

Syntex Chemicals, Inc. states that it is in compliance with, or on a schedule to be in compliance with, all emission requirements set forth in permits, consent degrees and administrative orders applicable to the manufacture of the drug substance intermediate chlorohydrin at its facilities in Boulder, Colorado, as well as emission requirements set forth in Federal, State, and local statutes and regulations applicable to the manufacturing process.

Syntex Chemicals, Inc.

Gerald L. Hoerig
President

3 FEB. 97.
Date

SELF CERTIFICATION OF ENVIRONMENTAL COMPLIANCE

To whom it may concern:

Hoffmann-La Roche Ltd. states that it is in compliance with, or on a schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the production of the drug substance Saquinavir base at its facilities in Basle, Switzerland, as well as emission requirements set forth in applicable federal, state, and local statutes and regulations applicable to the production of drug substance Saquinavir base at its facilities in Basle, Switzerland. The approval and the subsequent increase in production is not expected to affect compliance with current emission requirements or compliance with environmental laws.

Head of Pharma Operations
Manufacturing
Chemical Production
Hoffmann-La Roche Ltd.

Dr. W. Häusermann

Basel, April 15, 1997

Commitment

Hoffmann-La Roche Incorporated hereby commits to provide the FDA with an official copy of a Self Certification of Environmental Compliance, from R.P. Scherer GmbH, for the bulk manufacture of TRADE NAME™ (saquinavir) Soft Gelatin Capsules) as soon as it is available.

APPENDIX F

SYNTEX CHEMICALS, INCORPORATED

EMISSIONS PERMIT INFORMATION

Syntex Chemicals, Incorporated Emissions Permit Information

Unless otherwise noted, the permitting authority is the Colorado Department of Health and Environment.

Air:

Air emissions from the Syntex facility are permitted under Facility Permit Number 93BO833, which has no expiration date, as it will eventually be rolled into a Title V air operating permit for the site.

Wastewater:

Wastewater discharges from Syntex are regulated by City of Boulder Permit Number 009603, which expires January 25, 2001.

APPENDIX G

CITED REFERENCES

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

IND #:20-828

REVIEWER	: N. Battula
CORRESPONDENCE DATE	: 05-09-97
CDER RECEIPT DATE	: 05-12-97
REVIEW ASSIGN DATE	: 05-16-97
REVIEW COMPLETE DATE	: 10-05-97

SPONSOR: Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, New Jersey 07110

SUBMISSION REVIEWED: Original

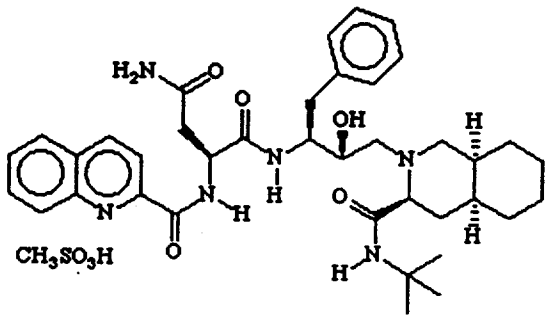
DRUG CATEGORY: Anti-HIV (protease inhibitor)

INDICATION: Treatment HIV infection in combination with other antiretroviral agents

DOSAGE FORM: Oral (soft gelatin Capsules, 200 mg)

PRODUCT NAMES:
a. PROPRIETARY: Fortovase
b. NONPROPRIETARY: Saquinavir
c. CHEMICAL: *cis*-N-*tert*-Butyl-decahydro-2[2(R)-hydroxy-4-phenyl-3(S)-{[N-(2-quinolylcarbonyl)-L-asparginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide methanesulfonate

STRUCTURAL FORMULA:



SUPPORTING DOCUMENTS:

Summary: This NDA is for approval of the drug product, Fortovase, by the FDA. The requested indication is based on virologic and immunologic surrogate markers. Fortovase is the soft gelatin capsule formulation of the drug substance saquinavir as free base and the drug product Invirase® is the hard gelatin formulation of the drug substance saquinavir as mesylate salt. Invirase® (NDA 20-628) in combination with nucleoside analogues was approved by the FDA on Dec 9, 1995 for the treatment of HIV infection.

Saquinavir is an inhibitor of HIV protease. HIV protease is an enzyme required for the proteolytic cleavage of viral polyprotein precursors into individual functional proteins found in infectious HIV. Saquinavir is a rationally designed peptidomimetic that binds to the active site of HIV protease and inhibits the activity of the enzyme. Inhibition of the HIV protease prevents cleavage of the viral polyproteins resulting in the formation of immature noninfectious HIV particles.

Bioavailability of Invirase®, the hard gelatin formulation of saquinavir was low (~4 %) and at the standard dose (600 mg tid) exposure to the active substance was sub-optimal in HIV infected individuals. In an effort to increase the bioavailability the sponsor designed fortovase, the soft gelatin formulation of saquinavir. The bioavailability of saquinavir in the soft gelatin formulation appears to be greater than the currently marketed hard gelatin formulation. The sponsor stated that combination of increased bioavailability of fortovase and the dose increase proposed (1200 mg tid) provides higher plasma exposure to the active substance and greater antiviral efficacy than the hard gelatin capsule.

This application does not directly contain microbiology data on the drug substance saquinavir. However, the sponsor referred to the microbiology data submitted with the initial approval of Invirase® (NDA 20-628). Subsequent to Invirase® approval, several publications appeared in the literature which evaluated the emergence of resistance in HIV against saquinavir and cross-resistance to other protease inhibitors. In addition, the sponsor submitted preliminary HIV resistance data from patient isolates treated with fortovase. Accordingly, the microbiology label is revised to reflect current evidence on saquinavir resistance and cross-resistance.

Conclusion: The microbiology portion of the draft label as currently written is acceptable (copy attached). With respect to microbiology this NDA is approved.

Recommendations for Phase 4 post approval studies:

Reference: 1. Lazdins, J.K., et al. J Infect Dis **1997;** 1063-1070

Narayana Battula

Narayana Battula, Ph.D.
Microbiologist

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20828

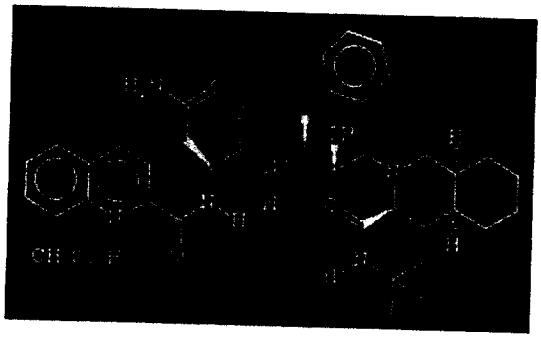
PHARMACOLOGY REVIEW(S)

PHARMACOLOGIST'S REVIEW

AUG 25 1997

NDA 20-828

Original NDA
Date Submitted: 5/12/97
Date Assigned: 5/14/97
Date Review Completed: 8/18/97
Reviewed by: Kuei-Meng Wu
HFD-530



SPONSOR:

Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, New Jersey 07110

DRUG:

FORTOVASE®, Saquinavir Soft Gel Capsule, Ro 31-8959/003 (mesylate salt, CAS# 149845-06-7), Ro 31-8959/000 (free base, CAS# 127779-20-8), cis-N-tert-Butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl] amino]butyl](4aS,8aS)-isoquinoline-3(S)-carboxamide methylsulfonate, Formula: $C_{38}H_{50}N_6O_5.1{:}1CH_4O_3S$; MW: 767 (free base = 671)

FORMULATION: Soft Gelatine Capsule (as the Inactive ingredients) 200 mg

INDICATIONS: for Use in Combination with Other Antiretroviral Agents in the Treatment of Hiv Infection When Therapy Is Warranted

INTRODUCTION

Saquinavir (Ro 31-8959) is an antiviral drug developed as an oral therapy for the treatment of HIV infection. The antiviral activity of saquinavir results from inhibition of the HIV proteinase enzyme. The hard gelatine capsule formulation of saquinavir (HGC) has a very low oral bioavailability in both animals and man (~2.5%, ~4% and <12% in rat, man and marmoset, respectively). This low bioavailability results from a combination of poor, and saturable absorption (<20% in rat, ~30% in man), and rapid/saturable, metabolism of the drug. To improve the oral bioavailability of saquinavir, the sponsor developed a new soft gelatin capsule formulation (saquinavir SGC) to replace the HGC under this new NDA. Saquinavir SGC

This supplemental NDA summarizes the preclinical safety profile of saquinavir SGC that was previously submitted under IND amendments no. 285, 294 and 300. The majority of the preclinical safety profile of saquinavir SGC is cross-referenced to NDA 20-268 (saquinavir HGC). However, additional bridging studies were completed (see below) using the new formulation. These bridging toxicity studies have been reviewed under amendments nos. 152, 229, 244, 250, and 253 of IND . This document provided an overall summary and comments on the distinct safety issues of saquinavir SGC.

SUMMARY OF PRECLINICAL SAFETY INFORMATION: SAQUINAVIR SGC

The following are a list of key preclinical toxicity studies conducted using saquinavir SGC.

	IND Submission No.
Repeat-Dose Chronic Toxicity Studies	
A. Rats.	
1. An Exploratory Seven-Day Oral (gavage) Toxicity and Toxicokinetic Study in Rats (Report No. W-142295, Roche Lab., USA, 4/4/95, Vol. 36.1).	*152*
B. Dogs.	
1. A four-week oral (capsule) toxicity and toxicokinetic study in dogs (Report No. W-138424, Roche Lab., USA, 6/23/95, Vol. 36.1)	*152*
2. An exploratory oral (capsule) ascending-dose toxicity study in dogs (Report No. W-138417, Roche Lab., USA, 6/2/95, Vol. 36.1).	*152*
3. A Four-Week Oral Combination Toxicity and Toxicokinetic Study of Ro 31-89591A12 (Saquinavir) and A-84538 (Ritonavir) in Dogs (Study No. 06799; 9/4/1996; Roche Products Ltd., Welwyn Garden City, UK)	*250*
4. A Thirteen-Week Oral Toxicity and Toxicokinetic Study with Degraded and Non-Degraded Ro 31-8959 Soft Gelatin Capsules in Dogs (Study No. 06772; 8/30/1996; Roche Products Ltd., Welwyn Garden City, UK).	*250*
Reproductive Toxicity Studies	
1. A Segment II Reprotoxicity Study of Ro 31-89591/A21 and Ro 31-89591/A29 in Rats (Study No. 06845; 9/10/1996; Roche Products Ltd., Nutley NJ, USA)	*253*
Mutagenicity and Genotoxicity Studies	
1. Chromosome analysis in cultured human peripheral blood lymphocytes treated with Ro 31-8959/A14-00 in the presence and absence of a metabolic activation system (research report No. B-165'319, Study No. 201 M 95; 4/15/1996; batch# 101)	*244*
2. Mutagenicity evaluation of Ro 31-8959/A14-00 in the AMES test (research report No. B-165490, Study No. 203 M 95; 1/23/1996; batch# 101)	*229*

The preclinical safety information is highlighted as follows.

(1) TARGET ORGAN/SYSTEM AND PROFILE OF TOXICITY

Oral Repeat-Dose Toxicity in Rats

The main toxicities of saquinavir mesylate explored in rats as reported in NDA 20-268 were liver and low white counts. In this NDA, oral administration of saquinavir to rats for 7 days (526, 1400 mg/kg/day) did not provide additional toxicity data because the plasma exposure (AUC_{0-8h}) to saquinavir was not significantly increased by the use of the

formulation relative to that produced by saquinavir mesylate suspended in 10% succinylated gelatin (see Table 1 below). It is concluded that (1) the rat is not an appropriate species to further explore additional toxicity information that might result from the higher drug concentration expected to be achieved by the new formulation, and (2) bridging studies in rats (e.g., general and reproductive toxicity or carcinogenicity) using the new formulation are not necessary, and no difference in toxicity profiles is expected between studies using the formulation and those (previously completed and the ongoing ones) using saquinavir mesylate.

Table 1.
Mean toxicokinetic parameters of two formulation of Saquinavir on Days 1 and 7 of the rat study

Sample Day	Dose Level of Saquinavir (mg/kg/day) [a]	Succinylated Gelatin Formulations		Formulations	
		C_{max} (ng/mL)	AUC_{0-8h} (ng.h/mL)	C_{max} (ng/mL)	AUC_{0-8h} (ng.h/mL)
Day 1	526	803	4618	1528	7337
Day 7	526	561	2601	888	3050
Day 1	1400	1075	6281	1124	6996
Day 7	1400	836	3313	944	2836

Oral Repeat-Dose Toxicity in Dogs

Primary preclinical safety evaluation of the new saquinavir formulation was conducted in dogs, contrasting that of the old formulation in marmosets and rats. Saquinavir SGC administered orally to dogs for up to 13-week's duration caused dose-related increases in the incidence of emesis and soft stools/diarrhea. In a 4-week study, the no observable adverse effect level was considered to be 270 mg/kg/day, which gave a mean plasma exposure (AUC_{24} μg.h/mL=86.45 μg.h/mL) up to 4-fold greater than that seen in patients treated with the standard clinical dose of 1200 mg saquinavir SGC t.i.d (20 μg.h/mL.)

Table 2.
Mean toxicokinetic parameters of Saquinavir SGC on Day 6 of the 4-week dog study

Dose Group	*Saquinavir mg/kg/day*	*C_{max} ng/mL*	*AUC_{0-24h} μg.h/mL*
1	*0 (placebo)*	-	-
2	*0 (capsule)*	-	-
3	*40*	*1459*	*6.51*
4	*100*	*3558*	*16.84*
5	*270*	*16070*	*86.45*
6	*700*	*13510*	*177.9*

Table 3.
Mean toxicokinetic parameters of two formulation of Saquinavir (average of Days 33 and 86) of the 13-week dog study

Dose Group	Formulation	Saquinavir mg/dog/day	C_{max} ng/mL	AUC_{0-24h} µg.h/mL
1	Ro 31-8959/A09	0 (placebo)	-	-
2	— —	0 (capsule)	-	-
3	Ro 31-8959/16	40	750	2.8
4	Ro 31-8959/16	100	3540	19.7
5	Ro 31-8959/16	300	8220	58.8
6	Ro 31-8959/12	300	10490	74.3

Ro 31-8959/16: Non-degraded saquinavir SGC; Ro 31-8959/12: Degraded saquinavir SGC.

Reductions in body-weight and food consumption were seen at the high-dose level (700 mg/kg/day) in the four-week study. Functional hepatotoxicity (raised ALT, AST and ALP) was seen at dose levels of 300 mg/kg/day and above. No histopathological changes in hepatic tissue are evident except in one male dog (700 mg/kg/day for 4-weeks; AUC_{24h} = 648 µg.h/mL) that showed scattered hepatocellular necrosis, bile duct proliferation, light-brownish pigment in Kupffer cells and vascular (sinusoidal) leukocytosis.

In summary, the studies conducted in the dog identified potential adverse effects of the new formulation in the gastrointestinal (GI) system and confirmed the liver as a target organ for the toxicity of saquinavir. These preclinical findings were similar to adverse events seen in clinical studies with saquinavir SGC and thus the dog is a predictive model for toxicity findings with this formulation in man.

(2) REPRODUCTIVE TOXICITY

An oral (gavage) study was conducted in pregnant Sprague-Dawley (Crl:CD) rats to determine the embryotoxic and teratogenic potential of degraded saquinavir Soft Gelatin Capsule (SGC) formulation. Doses of 0 (placebo control), 0 (water control), 50, 150 and 500 mg/kg/day of saquinavir in degraded formulation and 500 mg/kg/day of saquinavir in non-degraded formulation were employed. Degradation of saquinavir SGC formulation did not produce adverse effects on embryo-fetal growth or development in the rat. Total degradation products were administered at dose levels up to 17 times greater than the maximum that could be received by patients given SGC (3.6 g saquinavir/day). Dilated renal pelves and dilated/convoluted ureters were observed with its incidence reaching statistical significance in the treated groups when compared with placebo-control group, but not with the water-control group. The sponsor indicated that because of a lack of a dose-response relationship, and the equivalent rate with the water-control group, the findings are considered nontreatment-related. A reduced food consumption and/or reduced body-weight gain in most of the affected dams was also reported.

(3) MUTAGENICITY AND GENOTOXICITY STUDIES

Degraded saquinavir SGC formulation and alone showed no mutagenic or genotoxic activity *in vitro* in either the Ames test or the Human Chromosome Aberration assay. These studies were primarily conducted to qualify impurities/degradation products and no mutagenic or chromosome aberration were seen in these *in vitro* assays. alone was also negative in these studies.

(4) CARCINOGENICITY STUDIES

Saquinavir SGC, at a dose of 1200 mg t.i.d., produces plasma exposure of approximately 20 μg.h/mL in patients. The ongoing carcinogenicity studies, as originally intended to support the old formulation (saquinavir hard gelatine capsule), produces maximal plasma exposures ($AUC_{0\text{-}24h}$) of approximately 10 μg.h/mL in rats (doses chosen: 0, 125, 350 and 1000 mg/kg/day), and 24 μg.h/mL in mice (doses chosen: 0, 200, 700 and 2500 mg/kg/day.) Thus, very little or no safety margin exists relative to the ongoing carcinogenicity studies. The sponsor does not want to conduct further carcinogenicity tests with saquinavir SGC because (1) the new formulation does not increase systemic exposure to saquinavir in rats when compared to that achieved in previous studies, and (2) the SGC formulation could not be incorporated as a dietary admixture due to its relative instability compared to the mesylate salt.

(5) PHARMACOKINETICS

The sponsor cross-referenced all pharmacokinetic and toxicokinetic data to NDA 20-268. A complete ADME profile of *saquinavir mesylate* was discussed in the *Pharmacologist's Review* on NDA 20-268. Although *saquinavir SGC* increased the exposure of a 600 mg (10 mg/kg) dose to humans approximately 3 fold, the new single-dose and multi-dose pharmacokinetic studies conducted in rats using *saquinavir SGC* showed no significant increases in exposure (1.6 and 1.1 fold at 300 and 800 mg/kg). Similar new pharmacokinetic studies conducted in the dog showed variable, but often very high, exposure (see *Tables 2 and 3* above). The threshold for hepatotoxicity in this species was determined to be around 250 ug.h/ml ($AUC_{0\text{->}24hr}$), which is at least ten times the average exposure to patients given the standard 3 x 1200 mg daily dose of *saqinavir SGC*. The mechanism for the enhancement of bioavailability by the new SGC formulation was not fully investigated and not clearly understood. The sponsor speculated that a combination of partial absorption from the gut, P-glycoprotein-catalyzed secretion back into the lumen of the small intestine and CYP3A4-catalyzed metabolism in both the gut-wall and liver appears to be responsible.

(6) THE DEGRADANTS/IMPURITIES IN THE DRUG SUBSTANCE OR DRUG PRODUCT

The sponsor has identified seven organic impurities in the *drug substance* (saquinavir free base). They are Ro 31-9232, Ro 31-9485, Ro 32-3038, Ro 31-9533, Ro 32-0076, Ro 32-3103 and Ro 61-4520. In the *drug product* (saquinavir SGC), the impurities identified were Ro 31-9232, Ro 31-9485, Ro 32-0904, Ro 32-3038, Ro 32-5591, Ro 32-5592, Ro 64-0837. Although these drug-related chemicals are degraded from the saquinavir peptide, they do not retain any peptide- or amino acid-like properties. In addition to these identified degradants,

the *drug product* contains 0.3-1.1% of an impurity (RRT 1.23) whose structure is unknown. Preclinical toxicity studies using either the drug substance or drug products contained a similar or higher percentage of impurities than the specification limits (. For toxicity exploration purposes, higher percentage of impurities was intentionally generated by the sponsor using adverse ambient conditions (e.g., higher temperature≈50°C). In consideration of the low maximal daily dose level for an impurity/degradation product (1.08 mg/kg/day for a 50 kg person taking 3.6g saquinavir SGC) relative to that administered to animals without adverse effect and the patient population being treated, the levels for these impurities/degradation product are in general considered acceptable in terms of risk of adverse effects to HIV patients.

(7) RISK ASSESSMENT OF SAQUINAVIR SGC BASED ON PRECLINICAL TOXICITY DATA

There are two major preclinical toxicological issues related to this saquinavir SGC NDA. First, the new saquinavir formulation tends to degrade and the residues or impurities pose unknown toxicity profiles that have not been characterized in the previous saquinavir HGC NDA. Second, the bioavailability of the new formulation in humans increased significantly with drug exposure elevated by more than 10 times, as compared with the old formulation (20 ug.hr/ml vs. 2 ug.hr/ml). The oral toxicity and toxicokinetic studies on saquinavir SGC as conducted in the dog addressed these two issues satisfactorily. However, the studies conducted in the rat did not produced a sufficiently high exposure level to cover the clinical concentration, because of limited bioavailability of the new formulation in the rat. Thus, the data obtained from the rat did not provide additional insights into new safety concerns about the new formulation, particularly those related to reprotoxicity and carcinogenicity of saquinavir SGC while the supporting data were based on rat studies. No margin of safety on the risk assessment of these two toxicities could be expected (40-50% of human exposure) resulting from the 10-fold elevation in drug plasma levels. Because of the limitation of feasibility, these points has been addressed in the drug's label (see Appendix.) In regard to systemic toxicity, the dog data showed a moderate toxicity profile similar to that produced by the old formulation. In summary, the sponsor has addressed significant preclinical pham/tox issues related to saquinavir SGC and alleviated safety concerns about this NDA.

CONCLUSION

From the preclinical pharmacology/toxicology point of view, the bridging studies are sufficient to support the NDA on saquinavir SGC. No regulatory comments will be provided for this NDA.

KUEI-MENG WU, PH.D.
REVIEWING PHARMACOLOGIST
DAVDP

CONCURRENCES:
HFD-530/DEP DIR/WDEMPSEY 8/28/97
12/5/97

CC:
HFD-530 NDA 20-838(000)
HFD-530/DIVISION FILE
HFD-530/CSO/[redacted] KELLY
HFD-530/MO/JMURRAY
HFD-530/CHEM/PLIU